Data Evaluation Record on the Acute Contact and Oral Toxicity of Fluopyram (AE C656948) + Tebuconazole (HWG 1608) SC 400 G to Honeybees (*Apis mellifera*)

EPA MRID Number 47567613

Data Requirement: EPA DP Barcode: D386298
EPA Guideline: OPPTS 850.3020 (contact); non-guideline (oral)

Test material: FLU+TBZ SC200+200A G **Purity:** 17.1% w/w FLU, 17.5% w/w TBZ
Common name
Chemical name: Fluopyram (AE C656948) and Tebuconazole (HWG 1608)

Primary Reviewer: Stephen Carey, Biologist
EPA/OCSPP/OPP/EFED/ERB6

Signature: Steph Carey 7/28/11
Date: 7/28/11

Secondary Reviewer(s):
{EPA/OECD/PMRA}

Date:

Reference/Submission No.: {.....}

EPA PC Code 080302/128997

CITATION: Schmitzer, S. 2007. Effects of AE C656948 + Tebuconazole SC 200+200 g/L (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory. Unpublished study performed by Institut fuer Biologische Analytik und Consulting IBACON, Rossdorf, Germany. Laboratory Project Number: 34501035. Document No. M-291673-01-2. Report ID. EBGMP071. Study sponsored by Bayer CropScience AG, Frankfurt, Germany. Study completed August 14, 2007.

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Executive Summary:

The acute contact toxicity of Fluopyram + Tebuconazole SC 400 (200+200) g/L to bees (*Apis mellifera* L.; female worker bees) was studied in a 48 h test with 0 (formulation control), 12.5, 25, 50, 100 and 200.0 µg product/bee (nominal). The acute oral toxicity of Fluopyram + Tebuconazole SC 400 (200+200) g/L to bees was studied in a 48 h test with 0 (formulation control), 6.8, 13.2, 26.6, 54.2 and 108.3 µg product/bee (measured). For both tests, 3 replicates, each consisting of 10 bees in one cage per test concentration, were assessed for mortality after 4, 24 and 48 hours. Reference item was Dimethoate 400 g/L (nominal).

In the contact test, mortality occurred in all groups (except the 25.0 µg/bee group) dosed with fluopyram +tebuconazole SC 400 (200+200) g/L, increasing with dose levels. 3.3% mortality occurred in the control (water + 0.5% Adhäsit). During the first 4 hours behavioral impairments such as discoordinated movements and apathy were observed in the contact test at ≥50.0 µg product/bee dose levels. During the 24-hours assessment, these behavioral impairments were found in ≥100 µg/bee dose groups. No further behavioral abnormalities were observed at 48 hours.

Oral doses of 108.3, 54.2 and 6.8 µg product/bee led to mortalities of 100.0, 36.7 and 3.3%, respectively at the end of the test (48 hours after application). 6.7% mortality occurred in the control (50% sugar solution). In the oral test during the first 4 hours discoordinated movements and/or apathy were observed in the 3 highest dose levels. Afterwards, no more behavioral impairments occurred at any time in any of the test item treatments.

In the contact toxicity test the LD_{50} (24 h + 48 h) and NOAEC (based on 4 h) were > 200.0 and 25 µg product/bee, respectively. The 48-hr LD_{50} and NOAEC were 62.5 and 25 (based on 4 h) µg product/bee in the oral toxicity test, respectively.

The study is scientifically sound but does not satisfy the EPA guideline requirement for an insect pollinator acute contact study with honey bees; thus, without concentration measurements of the formulation product at test initiation and termination, the study is classified as SUPPLMENTAL. The portion of the acute oral toxicity study with honey bees is not an EPA guideline study and will be used as supplemental information.

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I) Material and Methods

Guideline Followed: OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test, (adopted 21st September 1998); OECD 214: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Contact Toxicity Test, (adopted 21st September 1998)

Compliance: The study was conducted in compliance with:

- The OECD Principles of GLP (revised in 1997) ENV/MC/CHEM(98)17
- Chemikaliengestz (Chemicals Act) der Bundesrepublik Deutschland (ChemG), Anhang 1 (Annex 1), 2002
- Directive 2004/10/EC of February 2004 (Official Journal No. L 50/44)

Which are consistent with:

- USEPA 40 CFR Part 160
- JMAFF, 11 Nousan, Notification No. 6283, Agricultural Production Bureau (October 1999)

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. Materials

1. Test material:	Fluopyram (AE C656948) + tebuconazole (HWG 1608) SC 200 + 200
Specification No:	102000016375
Batch No:	2007-002120
Purity:	Nominal: 200 g fluopyram/L + 200 g tebuconazole/L Analyzed: 201 g fluopyram /L (17.9 % w/w) and 200 g tebuconazole /L (17.8% w/w)
Visual appearance:	white suspension
Density:	1.123 g/mL

2. Vehicle and/or positive control

Control:	
Contact test:	tap water + Adhasit treated control (applied after anesthetization with CO2)
Oral test:	50% aqueous sugar solution (in tap water) tap water + Adhasit treated control (applied after anesthetization with CO2)
Reference substance:	Perfekthion EC, active substance: dimethoate, 414.4 g/L

(analysed).

Test treatment level:

Contact test:	0.30, 0.20, 0.15 and 0.10 ug Dimethoate per bee (nominal)
Oral test:	0.30, 0.15, 0.08 and 0.05 ug Dimethoate per bee (nominal) 0.32, 0.16, 0.09 and 0.06 ug Dimethoate per bee (measured)

Test results:

Contact test:	LD_{50} (24 h): 0.20 µg a.i./bee
Oral test:	LD_{50} (24 h): 0.13 µg a.i./bee

3. Test organism:

Species:	Honeybee - *Apis mellifera L.* (Hymenoptera, Apoidea)
Growth stage and Sex:	Female worker bees of a disease-free and queen-right colony.
Source:	Internal breeding of IBACON GmbH. Honey bee colonies, disease-free and queen-right, bred by IBACON

4. Environmental conditions:

Test Environment:	Incubator
Temperature:	25-26°C
Photoperiod:	Constant darkness except during observations
Relative humidity:	40 to 73%
Ventilation	Ventilation to avoid possible accumulation of pesticide vapour

B. Study design and methods

1. In life dates:	2007-04-16 to 2007-05-10 (both studies)
2. Experimental treatment and observations:	
Test duration:	48 hours
Test unit type:	Stainless steel chambers
Number of test levels:	1 control, 5 treatment concentrations
Applied concentrations:	
Contact test:	200.0, 100.0, 50.0, 25.0 and 12.5 µg product/bee (nominal)
Oral test:	100.0, 50.0, 25.0, 12.5 and 6.3 µg product/bee (nominal) 108.3, 54.2, 26.6, 13.2 and 6.8 µg product/bee (measured)
Number of bees:	10 per test unit
Number of replicates:	3 per test item dose level, controls and reference item doses
Diet:	Commercial ready-to-use syrup (Apiinvert; 30 % Saccharose,

31 % Glucose, 39 % Fructose) ad libitum; was supplied directly after treatment via syringes that were inserted into the cages via an opening on the top and from which bees accessed the food directly.

Exposure
Worker bees of the honeybee *Apis mellifera* L. were exposed to 200.0, 100.0, 50.0, 25.0 and 12.5 µg product/bee (nominal) in the acute contact test and to 108.3, 54.2, 26.6, 13.2 and 6.8 µg product/bee (measured) in the acute oral test. Perfekthion (a.s.: dimethoate 414.4 g/L) was used as reference item. The treated bees were kept under controlled climatic conditions and assessed for toxic effects for up to 48 hours. The cages (stainless steel cages with ventilation holes in the bottom and a glass plate in front for observation of the bees, dimensions inside: 10 cm x 8.5 cm x 5.5 cm) were ventilated to avoid possible accumulation of pesticide vapor. At the beginning of the test, 10 healthy worker bees per replicate (5 replicates/product, control and reference item) were transferred individually in glass tubes from the hive.

Food:
Commercial ready-to-use Apiinvert syrup containing 30% saccharose, 31% glucose and 39% fructose. Food was given ad libitum, immediately after applications.

Application in the contact test:
A single 5 µL droplet of Fluopyram + tebuconazole SC 400 (200+200) g/L in an appropriate carrier (tap water + 0.5% Adhäsit) was placed on the dorsal bee thorax using a Burkard – Applicator. For the control one 5 µL roplet of tap water containing 0.5% Adhäsit was used. The reference item was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.5% Adhäsit).

Application in the oral test:
Aqueous stock solutions were prepared and then mixed 1 + 1 with the ready-to-use syrup (100% sugar; 50% ready-to-use syrup; Apiinvert, Südzucker, D-97195 Ochsenfurt; content: 30% Saccharose, 31% Glucose, 39% Fructose) to achieve the required test concentrations and so that the final syrup solution was 50%. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake ranged from 0.75 to 2.5 hours for the test item treatments). After a maximum of 2.5 hours, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. The target dose levels (e.g. 100 µg product/bee nominal) is obtained if 20 mg/bee of the treated food was ingested. In practice, higher (or lower) dose levels were obtained as the bees had a higher or lower uptake of the test solutions than the nominal 20 mg/bee.

3. Observations:
Endpoints:
The number of dead and affected bees was counted at 4, 24 and 48 hours. During assessments times any behavioral abnormalities of the bees were also recorded: vomiting, apathy, intensive cleaning.

Statistical methods

The oral LD_{50} of the test item and the contact and oral LD_{50} of the reference item were estimated according to moving average computations (Thompson and Weil, 1952). The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925). The software used to perform the statistical analysis was ToxRat Professional, Version 2.09, ® ToxRat Solutions GmbH, © 2005.

I) Results and Discussion

Findings and observations

The results of the mortality and behavioural assessment for the oral and contact tests are presented in **Tables 1 and 2**, respectively.

Table 1: Reported mortality and behavioral abnormalities of the bees in the oral toxicity test (results are average from 3 replicates [10 bees each] per dosage/control)

Dosage [µg a.s./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]
Test item						
108.3	40.0	60.0	100.0	0.0	100.0	0.0
54.2	13.3	33.3	36.7	0.0	36.7	0.0
26.6	0.0	3.3	0.0	0.0	0.0	0.0
13.2	0.0	0.0	0.0	0.0	0.0	0.0
6.8	0.0	0.0	0.0	0.0	3.3	0.0
Water	0.0	0.0	0.0	0.0	6.7	0.0
Reference item						
0.32	73.3	26.7	96.7	3.3	100.0	0.0
0.16	20.0	30.0	93.3	6.7	96.7	0.0
0.09	0.0	3.3	16.7	0.0	20.0	0.0
0.06	0.0	0.0	0.0	0.0	3.3	0.0

Table 2: Reported mortality and behavioral abnormalities of the bees in the contact toxicity test (results are average from 3 replicates [10 bees each] per dosage/control)

Dosage [µg a.s./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]
Test item						
200.0	3.3	50.0	36.7	16.7	46.7	0.0
100.0	3.3	30.0	23.3	10.0	30.0	0.0
50.0	0.0	13.3	10.0	0.0	10.0	0.0
25.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2: Reported mortality and behavioral abnormalities of the bees in the contact toxicity test (results are average from 3 replicates [10 bees each] per dosage/control)

Dosage [µg a.s./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality	behav. abnorm.	mortality	behav. abnorm.	mortality	behav. abnorm.
	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]	mean [%]
12.5	0.0	0.0	0.0	0.0	3.3	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.30	20.0	43.3	100.0	0.0	100.0	0.0
0.20	3.3	16.7	46.7	3.3	50.0	0.0
0.15	0.0	0.0	13.3	0.0	23.3	0.0
0.10	0.0	0.0	3.3	0.0	10.0	0.0

Table 3: Reported Mortality in the oral test

	24 h	48 h
Test Item Oral LD_{50} [µg product/ bee]	58.3	62.5
95 %- Confidence limit (lower):	49.1	54.4
95 %- Confidence limit (upper):	69.2	71.7

Mortality in the contact test:
Since mortality in the highest dose group with 200.0 µg product/bee was < 50%, the contact LD50 can be considered as > 200.0 µg product/bee.

Behavioral abnormalities in the contact test:
During the first 4 hours behavioral impairments such as discoordinated movements and apathy were observed in the contact test at dose levels of 200.0, 100.0 and 50.0 µg/bee. During the 24-hours assessment, these behavioral impairments were found in the 200 and 100 µg/bee dose group. No further behavioral abnormalities were observed at 48 hours.

Behavioral abnormalities in the oral test:
During the 4 hours check, discoordinated movements and apathy occurred in the 3 highest dose levels. After 24 and 48 hours no further behavioral impairments were observed. There were behavioral abnormalities consistent with the observed toxicity in the reference item test.

II) Conclusion of study author:

The toxicity of AE C656948+Tebuconazole SC 200+200 g/L was tested in both an acute contact and oral toxicity test on honey bees. The LD_{50} (24 h + 48 h) was > 200.0 µg product/bee in the contact toxicity test, respectively. The LD_{50} (24 h + 48 h) was 58.3 and 62.5 µg product/bee in the oral toxicity test, respectively.

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EPA Reviewer's Conclusion:

The test concentrations of the formulation product in the contact test (an EPA guideline study) were not measured which affected the acceptability of the study.

The study authors did not report an NOAEC, the reviewer determined the NOAEC based on visual interpretation. Apathy and moving coordination problems were observed in the three highest test concentrations at 4-hours; the NOAEC was determined to be 25 µg product/bee for both contact and oral tests.

The Reviewer agrees with the following endpoints from the acute oral and contact toxicity study on honeybee *Apis mellifera* L. exposed to Fluopyram + tebuconazole SC 200 + 200:

48 h oral LD_{50} > 200 µg product/bee
48 h contact LD_{50} =62.5 µg product/bee
NOAEC (based on 4-hr observations) = 25 µg product/bee

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